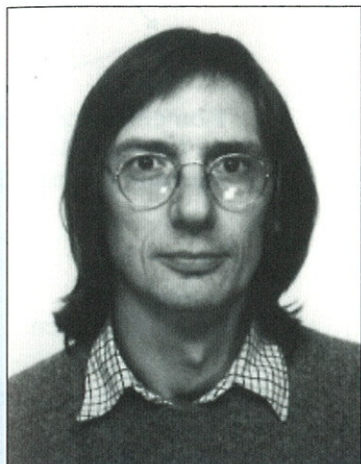


Zbigniew Ulanowski

Department of Physical Sciences, University of Hertfordshire,
Hatfield, Herts AL10 9AB, UK
E-mail: z.ulanowski@herts.ac.uk



Zbigniew Ulanowski was born in Poland, where he obtained a degree in applied physics at the Technical University of Lodz. In 1988 he gained a PhD from what was then Hatfield Polytechnic for a study entitled **Investigations of microbial physiology and cell structure using light scattering**, which demonstrated that the heat resistance of bacterial spores could be accounted for by protoplast dehydration. He continued working in light scattering, with special interest in the characterization of single particles, and this has motivated his interest in non-contact manipulation techniques. **Single-beam optical traps have been in use in the author's laboratory at the University of Hertfordshire in Hatfield since 1988, when an optical tweezers instrument was constructed that to the best of his knowledge was the first one using a diode laser, as well as being the earliest portable, self-contained system in existence.**



One of the most extraordinary inventions of the last two decades must surely be tweezers that use light to lift microscopic objects - a small-scale version of the traction beam of science fiction. Single beam optical trapping, as the technique is more accurately called, is increasing in importance as a method for non-contact manipulation of microparticles, cells and even molecules and as a tool for the measurement of piconewton forces in liquid media.

1. Introduction

Single beam optical trapping, often called laser or optical tweezers, is a non-contact technique for the manipulation of microscopic objects using forces present in a strongly focused laser beam (Ashkin *et al.*, 1986). Such forces are due to radiation pressure near the focus of the beam and they can produce stable, three-dimensional trapping with just one beam. A trap can be created simply by focusing laser light into a small spot using a microscope immersion objective. It was demonstrated early on that living cells (Ashkin *et al.*, 1987) and even organelles within intact cells (Ashkin *et al.*, 1990) could be held by near infrared laser beams without sustaining damage. When combined with an optical microscope, the technique offers highly selective means of moving small particles in liquids. There is now considerable interest in exploiting optical trapping for particle separation, transfer and concentration over fields as diverse as microbiology, molecular biology, medicine, materials science and physical chemistry. Some of the most promising applications are biological, where the technique can be used for cell, organelle, membrane or macromolecule trapping, isolation, sorting and manipulation. It is not unexpected, therefore, that optical trapping evokes fascination and enthusiastic response when it is demonstrated to an audience for the first time. This appeal also makes the technique valuable as an educational tool.

Even Kepler suspected that light could exert a force, an effect referred to as radiation pressure. It is now known that radiation pressure allows stars like the Sun to exist by counteracting the force of their own gravity. The shorter tail of Halley's comet is known to be composed of small dust particles being forced away from the Sun by radiation pressure (Britannica, 1999). However, the discovery of optical tweezers by Arthur Ashkin and co-workers at the Bell Labs (Ashkin *et al.*, 1986) came quite unexpectedly, despite the simplicity of the device. The inventor modestly admitted that the breakthrough, while being the result of years of painstaking research, was at least partly due to serendipity (Ashkin, 1994; 1997). The surprising fact about optical tweezers is that optical trapping forces can act opposite to, as well as in, the direction of propagation of the light.

2. Principle of operation

The explanation of laser tweezers depends on how far one wishes to delve into the physical detail, and can be very involved. However, remarkably, a satisfactory explanation can be obtained at the level of geometrical

optics (Ashkin, 1992). A useful example is that of a transparent, spherical particle or droplet with refractive index greater than that of the surrounding medium, and placed in a laser beam with a wavelength much smaller than the radius of the particle. The laser beam is sharply focused (usually by a microscope objective lens) to a spot with a diameter comparable to or smaller than the wavelength. The explanation hinges on the fact that photons have linear momentum - a vector quantity characterized by magnitude and direction. The latter changes when a photon changes direction, as when crossing an interface between two media of different refractive index. Since the total momentum is conserved, the difference between the initial and the final momentum is transferred to the medium. In other words, through changing the direction of the photons constituting a ray of light some momentum is imparted to the interface - as when bouncing balls off a surface. Since the rate of change of momentum is force, the result we seek is the number of photons per unit of time multiplied by the change of momentum of each individual photon.

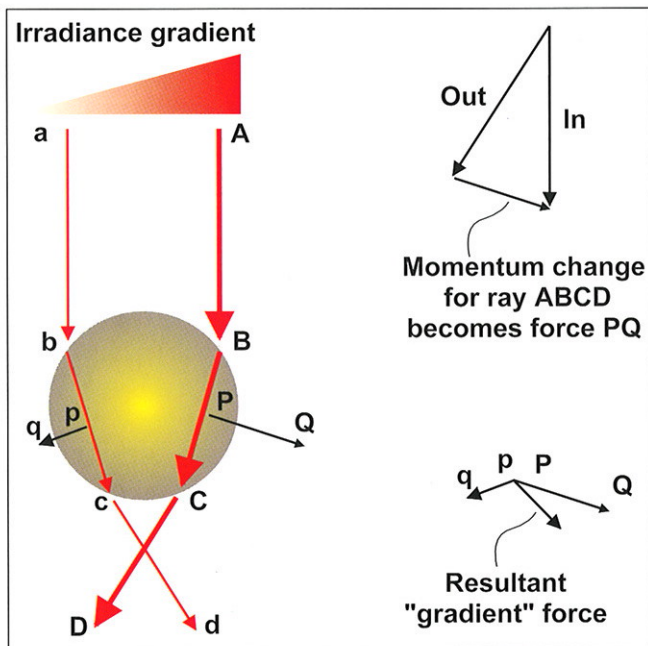


Fig. 1. Force diagram for a sphere in a beam containing a power density gradient represented by two rays of unequal power. **pq** and **PQ** are forces produced by the momentum transferred from the rays to the particle. The resultant force pulls the sphere towards the region of higher irradiance.

Thus, in Figure 1, a photon travelling along the path **abcd** imparts momentum to the spherical particle at **b** and **c**. The resultant is shown as vector **pq**. Similarly, two photons in the path **ABCD** of a stronger ray transfer momentum at **B** and **C**, the resultant being the twice-longer vector **PQ**. From this we see that the sphere is forced towards the region of more intense light - for a laser beam this usually means towards the beam axis. This force is often called the gradient force. Figure 2 shows the balance of force vectors for a particle positioned below the focal point of a focused beam directed downwards. We can see that the resultant

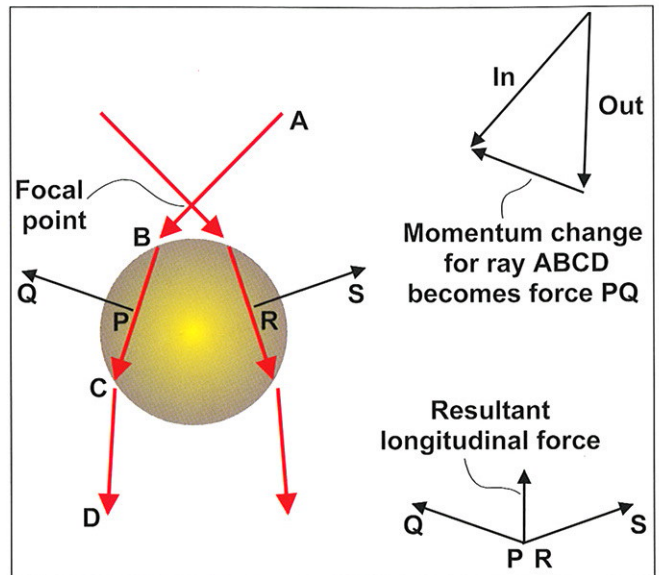


Fig. 2. Balance of force vectors for a spherical particle positioned below the focal point of a focused beam directed downwards and represented by two rays. **PQ** and **RS** are forces produced by the momentum transferred from the rays to the particle. The resultant force pulls the particle upwards.

force draws the particle upwards along the direction of propagation of the beam. This is the unexpected result: a particle can be pulled towards the source of light, against radiation pressure!

In reality, another, downward force is also present because some light is reflected off the particle (for clarity, the individual momentum vectors resulting from reflections are not shown in Figure 2 but can be sketched quite easily by considering the directions of reflected rays). As a result, the sphere resides in equilibrium a little beyond the focal point. This 'reflection force' is one reason why trapping is carried out on particles suspended not in air but in a liquid such as water. The refractive index of the particle is then reduced in relation to that of the external medium, resulting in smaller reflectivity. This in turn lowers the magnitude of the forward force component relative to the backward one and prevents the particle from being ejected from the trap. In air a counterbalancing force would be required to make the trap stable. If the beam of light is travelling upwards this force could be that of gravity - this is the principle of a related phenomenon called optical levitation.

A schematic diagram of a simple optical trap is shown in Figure 3. The apparatus is based on a microscope modified to transmit the laser beam and equipped with a video camera. A laser (with a power of a few to a few hundred milliwatts) produces a beam which is expanded using the lenses **L1** and **L2** prior to being reflected with a dichroic or semi-transparent beam splitter **BS** into the optical path of the microscope, where it is focused by an immersion objective **MO** into a sample on the stage **S**. The item to be trapped is suspended in water on a standard microscope slide. By carefully choosing the lenses **L1** and **L2** the beam can be

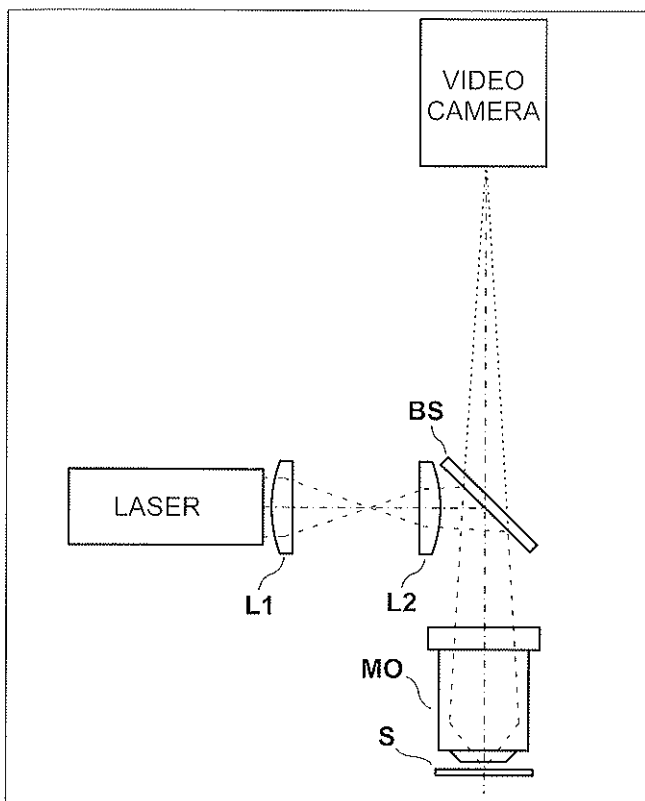


Fig. 3. Schematic diagram of optical tweezers. **L1, L2** - beam expanding lenses, **BS** - dichroic beam splitter transmitting visible radiation, **MO** - microscope objective, **S** - slide.

arranged to be focused near the image plane of the microscope and hence the beam spot and the trapped object can be viewed in the usual manner through the camera. The location of the spot can be changed by deflecting the beam and hence the object can be moved around on the slide. Alternatively, the whole stage could be displaced slowly, leaving the trapped object in the original position.

3. Applications

The number of applications of optical trapping is growing steadily and many hundreds of papers in this area have appeared in the 15 years since the original discovery. Consequently, the examples that follow are only a small cross-section of actual research and development activity.

Once an optical trap has been correctly set up, moving microscopic objects such as cells is extremely easy. Figure 4 shows a slide containing *Saccharomyces cerevisiae* (yeast) cells rearranged using optical tweezers into the shape of the letter H. When carried out using a computer mouse controlling the trap position and switching the laser on and off, an operation of this kind resembles dragging icons on a computer screen, and might take about a minute. Optical micro-manipulation can also be performed inside an intact living cell by focusing through the cell wall (Ashkin *et al.*, 1990). A pair of photographs recording the movement of an organelle within an algal filament is shown in Figure 5. Laser tweezers can enhance optical microscopy to allow

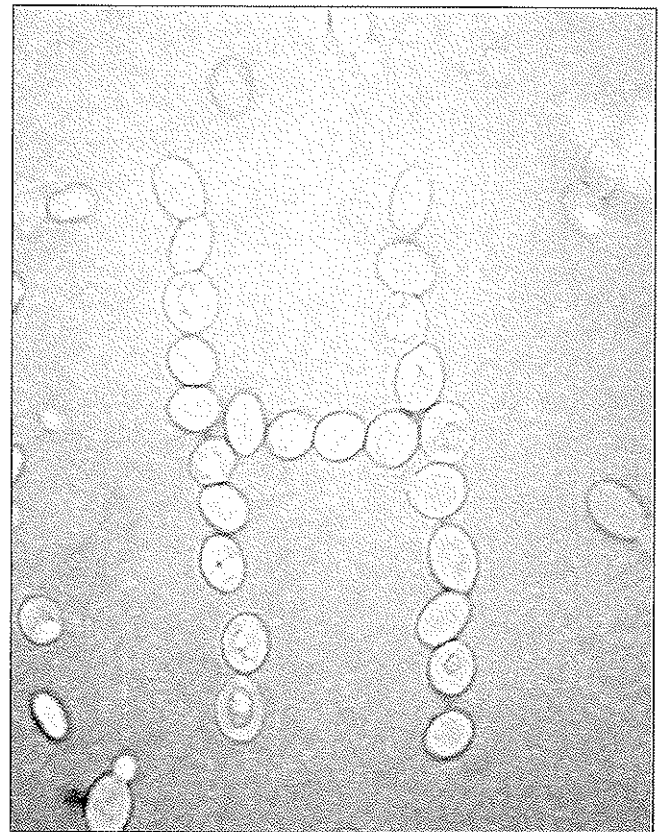


Fig. 4. *Saccharomyces cerevisiae* cells rearranged on a slide using optical tweezers. This operation took about a minute. For a video clip see <http://strc.herts.ac.uk/ls/ot.html>

interactive investigation of cell attachment to other cells or surfaces. Figure 6 contains a set of time-lapse images showing a cluster of three *S. cerevisiae* cells rotated by 360° around a precisely defined, constant point of attachment. In fact, the cluster could be rotated indefinitely without breaking the connection, suggesting that a single bond was involved. Short video clips illustrating the above procedures can be viewed on the world-wide web at <http://strc.herts.ac.uk/ls/ot.html>.

Optical trapping is growing in importance as a quantitative tool - for applying and measuring small forces

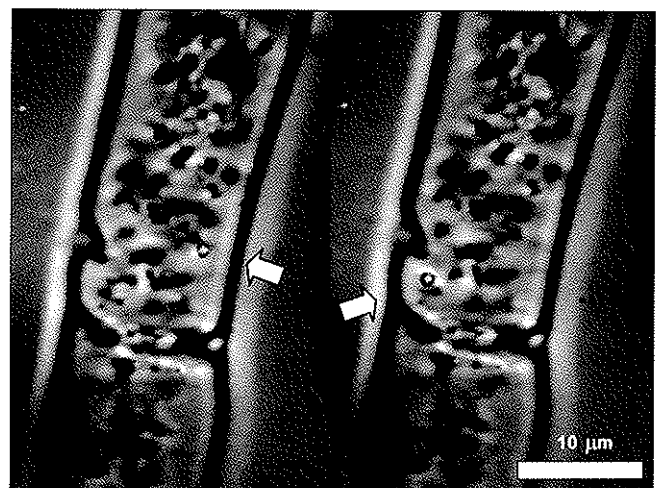


Fig. 5. Pair of photographs showing an organelle moved using optical tweezers within an intact filamentous cell of a green alga. The organelle (arrowed) originally visible near the right-hand side of the cell was transferred towards the left. For a video clip see <http://strc.herts.ac.uk/ls/ot.html>

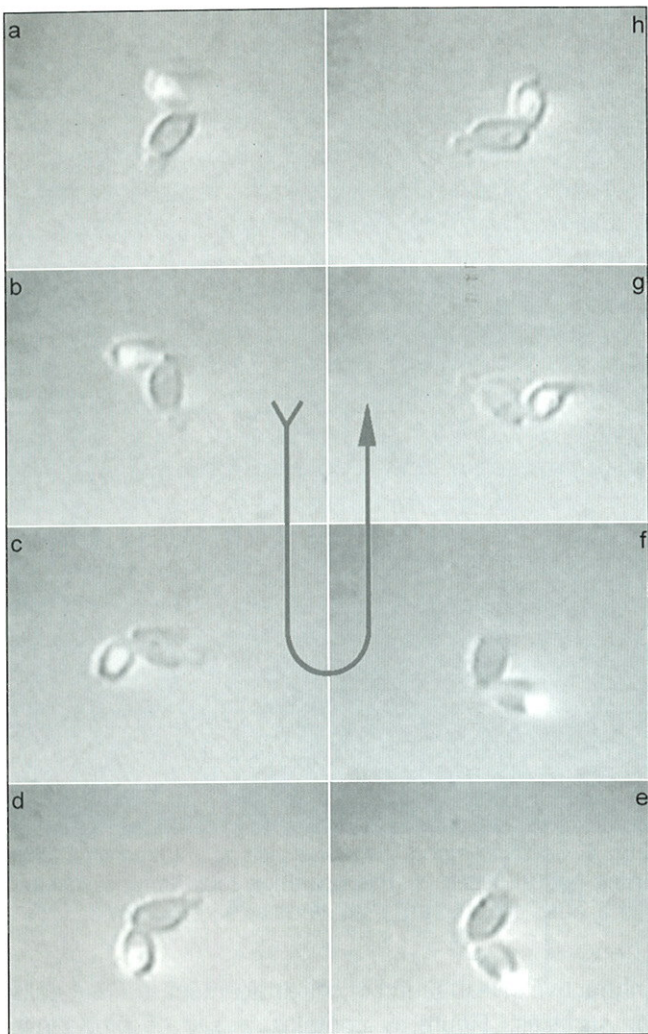


Fig. 6. Sequence of time-lapse images (a through to h) showing a cluster of budding *Saccharomyces cerevisiae* cells rotated using optical tweezers by 360° around a point of attachment to a microscope slide. The cluster could be rotated indefinitely without breaking the bond. The bright spot is the image of the laser beam. For a video clip see <http://strc.herts.ac.uk/ls/ot.html>

and torques, or as an enabling technique assisting a measurement process. Examples include studying cell elasticity (Bronkhorst *et al.*, 1995; Sleep *et al.*, 1999), response to applied pressure (Walker *et al.*, 1999) or cell motility and migration (Block *et al.*, 1989; Berry & Berg, 1997; Schwarzbauer, 1997). The interaction can be very selective - involving single molecules in some circumstances, as in the case of mechanoenzymes. In the latter area, where the measurement of piconewton forces generated by 'motor molecules' is required, optical trapping has already matured into a virtually indispensable research tool (Simmons *et al.*, 1996; Mehta *et al.*, 1998a). While it is necessary to bind the molecules onto larger 'handles', usually microspheres, for trapping to be possible, this actually allows an added level of selectivity because the surface of the objects acting as the handles can be chemically modified to accept a limited range of complementary molecules. Moreover, trapping forces acting on spherical objects can be precisely calibrated, typically using viscous drag (Sheetz, 1998; Svoboda & Block, 1994; Ulanowski & Ludlow, 2000).

In single macromolecule work optical trapping techniques have led to significant observations. For instance, reptation-type motion of a single, extended, fluorescently labelled DNA strand was demonstrated, as the molecule relaxed through a dense polymer solution (Perkins *et al.*, 1994). Upon stretching, DNA was found to yield abruptly to give an extended conformation almost twice the original length; the transition into the new form, now termed S-DNA, was reversible (Smith *et al.*, 1996; Cluzel *et al.*, 1996). A fascinating example of a previously impossible procedure is tying a 'molecular knot' with optical tweezers (Arai *et al.*, 1991). For reviews of single molecule studies see Mehta *et al.* (1998b) and Bustamante *et al.* (2000).

Optical trapping allows us to bring objects, such as cells of different type, into contact. This possibility opens up a host of applications in areas such as cell-cell and ligand-receptor interactions, immunology or, somewhat controversially, *in-vitro* fertilization (Bronkhorst *et al.*, 1997; Hoffmann, 1996; Seeger *et al.*, 1991; Sheetz, 1998; Zahn & Seeger, 1998). Potential exists for the construction of whole structures from small parts (Holmlin *et al.*, 2000), optionally followed by bonding, e.g. through photopolymerization (Mio & Marr, 1999; Misawa *et al.*, 1992). Such assembly can be aided by the use of multiple traps, either to hold and align larger objects or to manipulate several parts simultaneously (see next section). Unfortunately, parts micromachined from silicon cannot normally be held using standard single beam traps owing to high refractive index and optical absorption of this material, but this limitation does not apply to silica and similar substances.

Some of these applications can be assisted by the use of a companion technique - laser microsurgery, dubbed 'laser scissors' or 'laser scalpel' - somewhat inaccurately as the effect is more like that of a hole punch. In contrast to optical traps, a pulsed UV laser beam is used here, which can ablate a small volume of material, well defined in all three dimensions. Example applications include cell microsurgery (e.g. chromosome dissection), cell fusion in genetic engineering, insertion of objects into cells or 'zona-drilling' in *in-vitro* fertilization (Buer *et al.*, 1998; Greulich 1998; Greulich *et al.*, 2000; Hoffmann, 1996; Monajembashi *et al.*, 1997; Sheetz, 1998).

Another area where the optical trapping is becoming increasingly important is the isolation of previously unculturable microorganisms, such as bacteria (Mitchell *et al.*, 1993), yeast cells (Grimbergen *et al.*, 1993) and archaea (Huber *et al.*, 1995). Situations for which optical traps are uniquely suited occur where a microscopic object has to be maintained in isolation from solid supports, as in light scattering, spectroscopy and related measurements of microparticles, droplets, bubbles or other fragile structures such as micelles (Doornbos *et al.*, 1996; Gensch *et al.*, 1998; Grier, 1997; Urlaub *et al.*

al., 1996). Further areas of application include membrane properties (viscoelasticity, lateral diffusion etc.), cellular transport and the cytoskeleton (e.g. chromosome movement during cell division), scanning probe microscopy - for reviews see Ashkin (1997), Greulich (1998), Hoffmann (1996), Khan & Sheetz (1997), Monajembashi *et al.* (1997), Sheetz (1998).

Consequently, optical trapping can be expected to become a routine measurement and manipulation technique in many specialist applications within research and analytic laboratories. However, there are also prospects for the application of optical trapping as a general-purpose microscope attachment which can change passive observation into a more active, manipulative examination, possibly combined with quantitative force transduction. Examination of many types of microscopic specimen can be aided by the ability to rearrange and orientate objects either in one plane or in three dimensions. An optical trap makes viewing an object from more than one direction possible. It also allows testing particle attachment to surfaces, controlled deposition or bringing microscopic objects into contact (for example cells, labelled microbeads or micromachined particles), manipulation of organelles within cells and immobilisation of motile cells. The potential for use of optical trapping in education, for instance in demonstrations taking advantage of video microscopy, is also significant but it remains unexplored (Ulanowski & Williams, 1996). The value of this technique as an educational aid is enhanced by the fascination it almost invariably invokes due to its unusual nature.

4. Optical trap construction

One of the barriers preventing broader application of optical trapping, for example in education, is the cost of commercially available equipment. Furthermore, certain specialist applications require features not available in the few commercial designs currently on the market. For either reason, many potential users decide to construct their own trapping systems - see Figure 7 for an example. While more advanced designs can be very complex, basic optical tweezers are not difficult to build if one has some knowledge of optics and a few rules are observed. Constructing force measuring tweezers for single molecule work, although only slightly more complex, is outside the scope of this review - the reader is referred to publications by Sheetz (1998), Simmons *et al.* (1996) and Svoboda & Block (1994) for further details.

4.1. General principles

Operation of optical traps depends on the ability to focus light into a very small, near diffraction-limited spot, almost invariably using a high numerical aperture lens. In turn, efficient concentration of light requires the

utilisation of the whole aperture of the focusing lens. The diameter of the spot is approximately inversely proportional to the diameter of the focusing lens or the beam prior to focusing, whichever is smaller, and directly proportional to the focal length of the lens. This means that the numerical aperture must be high and the beam should fill the lens well if an efficient optical trap is to be created. A corollary is that if beam scanning is used, the beam ought always to point near the centre of the aperture of the objective (Escandon *et al.*, 1994; Svoboda & Block, 1994; Ulanowski & Ludlow, 2000).

Furthermore, if the objective to be used is characterized by finite conjugate ratio (finite tube length), a (parallel) laser beam must be made divergent so as to adapt it to the microscope. This last point cannot be overemphasized, as optical trapping demands that optical aberrations be kept to a minimum, and high numerical aperture microscope objectives are very sensitive to the placement of the conjugate image and object planes, as embodied by the microscope 'tube length' parameter. Efficient trapping cannot be expected if a parallel beam is directed through a typical microscope objective, of course with the exception of infinity-optics objectives. Moreover, the optimum tube length for trapping is likely to be different from the one for which the given objective was designed if infrared light is used. If the difference is significant and trapping is impaired at normal tube length, a supplementary 'tube lens' can be



Fig. 7. Microscope with built-in optical trap, constructed at University of Hertfordshire. The tracker ball visible in the lower right corner is used to control the position and intensity of the trapping spot. Above it is a unit interfacing the tracker ball (or mouse) to the trap and a laser driver, with a rheostat on top.

inserted between the dichroic mirror and the image plane to alter the optical tube length while maintaining the *mechanical* tube length. An unfortunate consequence of this will be a degradation of the visible image.

4.2. Microscope

A convenient basis for constructing optical tweezers is an optical microscope, except in rare specialist applications (Greulich, 1998; Mehta *et al.*, 1998a; Sheetz, 1998). The laser beam can be introduced into the optical path using an epifluorescence or camera port, after inserting an appropriate dichroic mirror (Figure 3). Unfortunately, this may involve having to replace lenses within the microscope in order to reduce beam aberrations to an acceptable level and minimize reflections - the beam is usually infrared! Failing that, an aperture may have to be cut into the body close to the nosepiece - if a dichroic mirror can be accommodated. Infinity-optics microscopes can be easier to adapt in this way owing to larger internal distances. An alternative design involves coupling the beam through the eyepiece (Afzal & Treacy, 1992).

The microscope should be equipped with a high numerical aperture objective, even if high magnification is not required, for reasons given above. In some applications, such as cell isolation, instead of the more usual oil immersion objective a water immersion one may be required, but the latter is usually expensive. One notable exception is the LOMO OM-25 objective (LOMO, St Petersburg, Russia) with a numerical aperture $NA = 1.23$ and a magnification of $\times 70$. This water immersion lens can be operated without a cover glass (although it is not designed for this), in effect giving a working distance of up to about $200 \mu\text{m}$. The cover glass thickness adjustment can be accomplished by using the built-in correction ring of the objective or by increasing the tube length of the microscope, or both (Ulanowski & Ludlow, 2000).

4.3. Laser

Viable trapping of biological materials such as cells requires the use of near infrared light (König, 1998; Sheetz, 1998; Svoboda & Block, 1994). The first near infrared optical traps were constructed using large and expensive solid-state lasers such as Nd-YAG ones. However, it is possible to use much less costly diode lasers if optical powers not exceeding 200 mW are required (Afzal & Treacy, 1992; Bakker Schut *et al.*, 1993; Escandon *et al.*, 1994; Doornbos *et al.*, 1996; Sato *et al.*, 1991; Ulanowski & Ludlow, 2000). The author has successfully used a variety of near infrared (780-850 nm), index guided, single transverse mode laser diodes with powers ranging from 2 to 150 mW. These included SDL-5300 and SDL-5400 series devices (SDL, Santa Barbara, CA, USA) and Philips 'col-

limator pens' (these latter devices have a built-in collimator). There is some evidence that wavelengths greater than 800 nm induce less damage to living cells, especially at higher power levels, hence lasers operating in the 820-850 nm region may be preferable (König, 1998). The laser should be connected to an adjustable driver usually operating in 'constant current' mode (this prevents laser power changes due to optical feedback, which might take place in 'constant power' mode). OEM-type drivers, rather than bench-top ones, are an economical choice in this context. It must be remembered, however, that diode lasers are sensitive devices and must be well protected from current transients and static electricity during operation and handling, necessitating the use of purpose-designed driver circuits. The laser mount should incorporate a heatsink and optionally a small fan, especially if the laser assembly is mounted within an enclosure. After collimation (by a special diode-laser collimator lens) the beam from a diode laser usually has an elliptical cross-section. While this makes the trap forces slightly asymmetric (Escandon *et al.*, 1994), beam circularization (e.g. using anamorphic prisms) is likely to be unnecessary as long as the trapped particles remain larger than the spot (Ulanowski & Ludlow, 2000).

4.4. Beam steering

Trapping spot positioning within the field of view of the microscope is often accomplished using beam-deflection devices such as piezoelectric or galvanometer controlled mirrors or acousto-optic modulators. The high cost and optical complexity of such an arrangement is justified if fast response is required, for example when forces produced by mechanoenzymes are to be measured (Greulich, 1998; Mehta *et al.*, 1998a; Sheetz, 1998; Simmons *et al.*, 1996) or if multiple traps are generated (see below). An alternative is to use linear slides driven by stepper or servo-motors in conjunction with a movable lens (Svoboda & Block, 1994), as in a recently described design suitable for use with diode lasers (Ulanowski & Ludlow, 2000). It is not too difficult to interface motors to a pointing device such as a tracker ball or mouse - see Figure 7. The system can be simplified in some cases by the use of manual, micrometer-driven positioners instead of motorized ones but a disadvantage of this option is, apart from operator discomfort, that manual positioning may transmit vibrations to the microscope and disturb the trapped object.

A promising development is the formation of multiple traps from a single laser beam using fast scanning (Visscher *et al.*, 1993; Mio & Marr, 1999). In this mode of operation the beam spot is rapidly switched between several locations, generating separate trapping regions. If the switching is sufficiently fast, viscous drag and inertia prevent trapped particles from following the spot as it moves between the separate locations - it is not even necessary to turn the beam off during

the transition periods. However, the technique requires the use of expensive beam scanners and complex driving electronics under computer control. An alternative approach uses a liquid-crystal spatial light modulator to generate the trapping beams 'in parallel', instead of sequential beam scanning (Mogensen & Gluckstad, 2000; Liesener *et al.*, 2000).

5. Conclusions

The traction beam has become a reality, albeit on a microscopic scale. The unusual properties of optical trapping and the precise control over small particles, cells and even molecules offered by this technique have attracted experimentalists in many areas of science, especially in biology. Many inventive and previously impossible experiments have been carried out, often with significant and important results. Consequently, optical trapping can be expected to become a routine measurement and manipulation technique in many specialist applications within research and even analytic laboratories. We can also anticipate a general-purpose microscope attachment, which can change passive observation into active, manipulative examination. The nature of this technique can convert a microscope with trapping capability into a valuable educational tool, although this latter application in particular awaits the emergence of affordable commercial instruments. Which ever way, there is laser light on the horizon!

References

- Afzal, R.S. & Treacy, E.B. (1992) Optical tweezers using a diode-laser. *Rev. Sci. Instrum.* **63**, 2157-2163.
- Arai, Y. Yasuda, R. & Itoh, H. (1991) Tying a molecular knot with optical tweezers. *Nature* **399**, 446-448.
- Ashkin, A., Dziedzic, J.M., Bjorkholm, J.E. & Chu, S. (1986) Observation of a single-beam gradient force optical trap for dielectric particles. *Opt. Lett.* **11**, 288-290.
- Ashkin, A., Dziedzic, J.M. & Yamane, T. (1987) Optical trapping and manipulation of single cells using infrared-laser beams. *Nature* **330**, 769-771.
- Ashkin, A., Schutze, K., Dziedzic J.M., Euteneuer, U. & Schliwa, M. (1990) Force generation of organelle transport measured *in vivo* by an infrared laser trap. *Nature* **348**, 346-348.
- Ashkin, A. (1992) Forces of a single-beam gradient laser trap on a dielectric sphere in the ray optics regime. *Biophys. J.* **61**, 569-582.
- Ashkin, A. (1994) *Rank Symposium on Optical Tweezers*. Grasmere, UK.
- Ashkin, A. (1997) Optical trapping and manipulation of neutral particles using lasers. *Proc. Natl. Acad. Sci. USA* **94**, 4853-4860.
- Bakker Schut, T.C., Schipper, E.F., de Grooth, B.G. & Greve, J. (1993) Optical-trapping micromanipulation using 780 nm diode-lasers. *Opt. Lett.* **18**, 447-449.
- Berry, R.M. & Berg, H.C. (1997) Absence of a barrier to backwards rotation of the bacterial flagellar motor demonstrated with optical tweezers. *Proc. Natl. Acad. Sci. USA* **94**, 14433-14437.
- Block, S.M., Blair, D.F. & Berg, H.C. (1989) Compliance of bacterial flagella measured with optical tweezers. *Nature* **338**, 514-518.
- Britannica (1999) Britannica CD. Encyclopaedia Britannica, Inc.
- Bronkhorst, P.J.H., Grimbergen, J. & Sixma, J.J. (1997) The mechanism of red cell (dis)aggregation investigated by means of direct cell manipulation using multiple optical trapping. *Br. J. Haematol.* **96**, 256-258.
- Bronkhorst, P.J.H., Streekstra, G.J., Grimbergen, J., Nijhof, E.J., Sixma, J.J. & Brakenhoff, G.J. (1995) A new method to study shape recovery of red blood cells using multiple optical trapping. *Biophys. J.* **69**, 1666-1673.
- Buer, C.S., Gahagan, K.T., Swartzlander, G.A. & Weathers, P.J. (1998) Insertion of microscopic objects through plant cell walls using laser microsurgery. *Biotechnol. Bioeng.* **60**, 348-355.
- Bustamante, C., Smith, S.B., Liphardt, J. & Smith, D. (2000) Single-molecule studies of DNA mechanics. *Curr. Opinion Struct. Biol.* **10**, 279-285.
- Cluzel, P., Lebrun, A., Heller, C., Lavery, R., Viovy, J., Chatenay, D. & Caron, F. (1996) DNA: An extensible molecule. *Science* **271**, 792-794.
- Doornbos, R.M.P., Schaeffer, M., Hoekstra, A.G., Sloot, P.M.A., de Grooth, B.G. & Greve, J. (1996) Elastic light-scattering measurements of single biological cells in an optical trap. *Appl. Opt.* **35**, 729-734.
- Escandon, G.J., Liu, Y., Sonek, G.J. & Berns, M.W. (1994) Beam magnification and the efficiency of optical trapping with 790 nm AlGaAs laser diodes. *IEEE Photon. Technol. Lett.* **6**, 597-600.
- Gensch, T., Hofkens, J., van Stam, J., Faes, H., Creutz, S., Tsuda, K., Jerome, R., Masuhara, H. & de Schryver, F.C. (1998) Transmission and confocal fluorescence microscopy and time-resolved fluorescence spectroscopy combined with a laser trap. *J. Phys. Chem. B* **102**, 8440-8451.
- Greulich, K.O. (1998) *Micromanipulation by Light in Biology and Medicine*. Birkhauser, Basel.
- Greulich, K.O., Pilarczyk, G., Hoffmann, A., Meyer Zu Horste, G., Schafer, B., Uhl V. & Monajembashi, S. (2000) Micromanipulation by laser microbeam and optical tweezers: from plant cells to single molecules. *J. Microsc.* **198**, 182-187.
- Grier, D.G. (1997) Optical tweezers in colloid and interface science. *Curr. Opinion Coll. Interface Sci.* **2**, 264-270.
- Grimbergen, J.A., Visscher, K., de Mesquita, D.S.G. & Brakenhoff, G.J. (1993) Isolation of single yeast-cells by optical trapping. *Yeast* **9**, 723-732.
- Hoffmann, F. (1996) Laser microbeams for the manipulation of plant-cells and subcellular structures. *Plant Sci.* **113**, 1-11.

- Holmlin, R. E., Schiavoni, M. & Whitesides, G.M. (2000) Light-driven microfabrication: assembly of multicomponent, three-dimensional structures by using optical tweezers. *Angew. Chemie* **39**, 3503-3505.
- Huber, R., Burggraf, S., Mayer, T., Barns., S.M., Rossnagel, P. & Stetter, K.O. (1995) Isolation of a hyperthermophilic archaeum predicted by in-situ RNA analysis. *Nature* **376**, 57-58.
- Khan, S. & Sheetz, M.P. (1997) Force effects on biochemical kinetics. *Ann. Rev. Biochem.* **66**, 785-805.
- König, K. (1998) Laser tweezers are sources of two-photon excitation. *Cell. Molec. Biol.* **44**, 721-733
- Liesener, J., Reicherter, M., Haist, T. & Tiziani H.J. (2000) Multi-functional optical tweezers using computer-generated holograms. *Opt. Commun.* **185**, 77-82.
- Mehta, A.D., Finer, J.T. & Spudich, J.A. (1998a) Use of optical traps in single-molecule study of nonprocessive biological motors. *Meth. Enzymol.* **298**, 436-459.
- Mehta, A.D., Pullen, K.A. & Spudich, J.A. (1998b) Single molecule biochemistry using optical tweezers. *FEBS Lett.* **430**, 23-27
- Mio, C. & Marr, D.W.M. (1999) Tailored surfaces using optically manipulated colloidal particles. *Langmuir* **15**, 8565-8568.
- Misawa, H., Sasaki, K., Koshioka, M., Kitamura, N. & Masuhara, H. (1992) Multibeam laser manipulation and fixation of microparticles. *Appl. Phys. Lett.* **60**, 310-312.
- Mitchell, J.G., Weller, R., Beconi, M., Sell, J. & Holland, J. (1993) A practical optical trap for manipulating and isolating bacteria from complex microbial communities. *Microb. Ecol.* **25**, 113-119.
- Mogensen, P.C. & Gluckstad, J. (2000) Dynamic array generation and pattern formation for optical tweezers. *Opt. Commun.* **175**, 75-81.
- Monajembashi, S., Hoyer, C. & Greulich, K.O. (1997) Microbeams and optical tweezers. *Microsc. Anal.* **81**, 7-9.
- Perkins, T.T., Smith, D.E. & Chu, S. (1994) Direct observation of tube-like motion of a single polymer-chain. *Science* **264**, 819-822.
- Sato, S., Ohyumi, M., Shibata, H., Inaba, H. & Ogawa, Y. (1991) Optical trapping of small particles using a 1.3 μm compact InGaAsP diode-laser. *Opt. Lett.* **16**, 282-284.
- Schwarzbauer, J.E. (1997) Cell migration. May the force be with you. *Curr. Biol.* **7**, R292-R294.
- Seeger, S., Monajembashi, S., Hutter, K.J., Futterman, G., Wolfrum, J. & Greulich, K.O. (1991) Application of laser optical tweezers in immunology and molecular-genetics. *Cytometry* **12**, 497-504.
- Sheetz, M.P. (ed.) (1998) *Laser Tweezers in Cell Biology. Methods in Cell Biology* **55**, Academic Press, San Diego.
- Simmons, R.M., Finer, J.T., Chu, S. & Spudich, J.A. (1996) Quantitative measurements of force and displacement using an optical trap. *Biophys. J.* **70**, 1813-1822.
- Sleep, J., Wilson, D. & Gratzner, W. (1999) Elasticity of the red cell membrane and its relation to hemolytic disorders: an optical tweezers study. *Biophys. J.* **77**, 3085-3095.
- Smith, S.B., Cui, Y. & Bustamante, C. (1996) Overstretching B-DNA: The elastic response of individual double-stranded and single-stranded DNA molecules. *Science* **271**, 795-798.
- Svoboda, K. & Block, S.M. (1994) Biological applications of optical forces. *Ann. Rev. Biophys. Biomol. Struct.* **23**, 247-285.
- Ulanowski, Z. & Williams, I.R. (1996) Optical tweezers. *Phys. Education* **31**, 179-182.
- Ulanowski, Z. & Ludlow, I.K. (2000) Compact optical trapping microscope using a diode laser. *Meas. Sci. Technol.* **11**, 1778-1785.
<http://www.iop.org/Journals/mt#featured>
- Urlaub, E., Lankers, M. & Kiefer, W. (1996) Applications of the optical trapping technique to analyze chemical reactions in single emulsion particles. *Fresenius J. Anal. Chem.* **355**, 329-333.
- Visscher, K., Brakenhoff, G.J. & Krol, J.J. (1993) Micromanipulation by multiple optical traps created by a single fast scanning trap. *Cytometry* **14**, 105-114.
- Walker, L.M., Holm, A. & El Haj, A.J. (1999) Mechanical manipulation of bone and cartilage cells with 'optical tweezers'. *FEBS Lett.* **459**, 39-42.
- Zahn, M. & Seeger, S. (1998) Optical tweezers in pharmacology. *Cell. Molec. Biol.* **44**, 747-761.